THE ROLE OF ANTIBODIES TARGETING *PLASMODIUM* SPOROZOITES: IS THEIR GREATEST IMPACT AT THE INOCULATION SITE?

by

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ABSTRACT

Malaria-causing *Plasmodium* parasites are deposited into host skin as infected *Anopheles* mosquitoes search for blood. In order for *Plasmodium* to establish infection in the liver, sporozoites need to exit the inoculation site, which they do by moving in the skin to find blood vessels and enter the circulation. This stage of the *Plasmodium* lifecycle (the pre-erythrocytic stage), at which parasite numbers are the lowest, has been recognized as a bottleneck for the parasite. RTS,S, the only vaccine candidate to have shown efficacy in Phase III clinical trials, targets the pre-erythrocytic stages of the parasite. Indeed further studies have shown that antibodies targeting the major surface protein of sporozoites (circumsporozoite protein or CSP) are critical for RTS,S-mediated immunity. We hypothesized that since sporozoites are extracellular for a significant period of time at the inoculation site, antibodies in the skin could contribute significantly to decreasing sporozoite infectivity. Using rodent malaria parasite *Plasmodium berghei*, we standardized the dose of sporozoites delivered intravenously and by mosquito bite that result in comparable liver infection. We then compared the efficacy of two different doses of a monoclonal antibody (mAb) specific for the *P. berghei* CSP repeats (50 µg & 25 µg mAb 3D11; IgG1) in their ability to inhibit infection when sporozoites were inoculated intravenously versus by mosquito bite. Our data shows that both concentrations of antibody have greater efficacy when sporozoites are inoculated by mosquito bite. These results have important implications for malaria vaccine development, and provide further insight into host-pathogen interactions in the skin.

Primary Reader: Photini Sinnis, M.D.

Secondary Reader: Fidel Zavala, M.D

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INTRODUCTION

Malaria Epidemiology & Control

Malaria is a mosquito-borne parasitic disease that has plagued humanity for centuries, and continues to exert a heavy disease burden, particularly in sub-Saharan Africa and Southeast Asia. According to the WHO Malaria World Report 2016, there were about 400,000 deaths reported from malaria – most of them in children.[1] This equates to roughly one child dying every three minutes. Moreover, an estimated two million people are at risk of contracting malaria.[1]

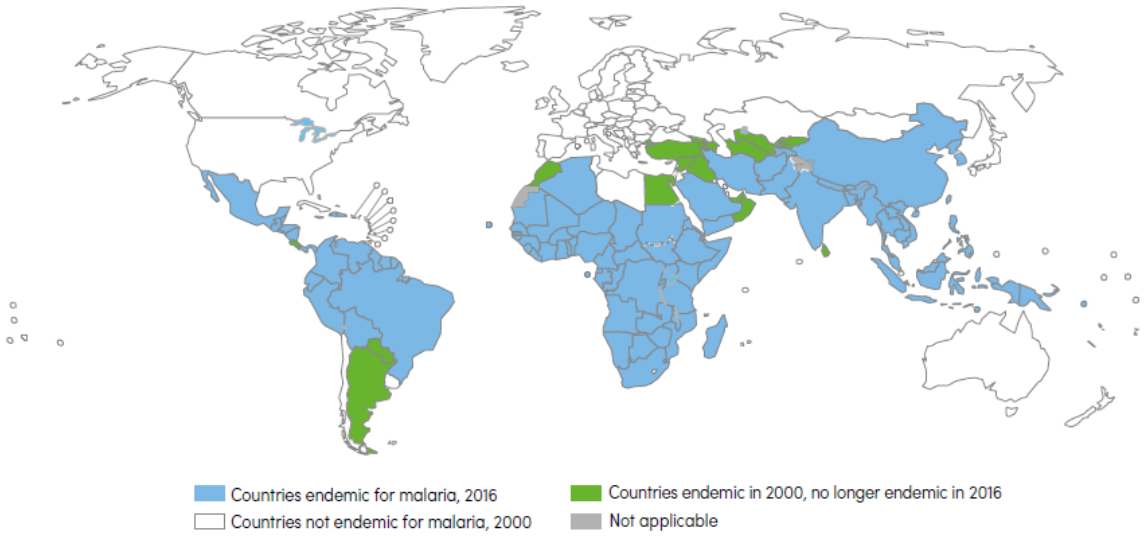


Figure 1. A schematic representation of the disease burden of malaria 2000-2016. Image credit: WHO World Malaria Report 2016[1]

The total burden of disease associated with malaria has decreased significantly over the past century. This has been achieved primarily through widespread use of

insecticide-treated bednets, mass campaigns for indoor residual spraying, and the availability of effective antimalarials combined with improved rapid diagnostics.[2,3] Although these tools have helped us gain a significant advantage in our continuing fight against malaria, it has become clear that eliminating, and subsequently eradicating, malaria will require additional tools including improved rapid diagnostics, novel therapeutic drugs, and effective vaccines.[4,5]

With an integrated approach to malaria elimination, it is hoped that we will be able to meet WHO's Sustainable Development Goals (SDGs) target of globally eliminating malaria by 2030.[6] However, the timely attainment of this goal has recently been threatened by the emergence of drug-resistant malaria in the Greater Mekong Sub-region (which includes Cambodia, the Lao People's Democratic Republic, Myanmar, Thailand and Vietnam).[3] A key tool in achieving these goals might be an effective malaria vaccine, preventing the establishment of infection and reducing the generation of drug-resistant parasites. The ensuing literature review will describe the malaria parasite life cycle, focusing in particular on the silent stage of infection – the pre-erythrocytic stage i.e. the stage of the parasite life cycle prior to clinical disease. This is followed by a discussion of RTS,S, arguably a milestone in malaria vaccine development but with considerable shortcomings and opportunities for improvement. Finally, the development of a novel methodology for studying the pre-erythrocytic stages of *Plasmodium* is discussed in the context of antibody-mediated immunity against malaria.

Plasmodium lifecycle & inoculation by mosquito

Malaria infection is initiated with the bite of an infected female *Anopheles* mosquito. While there are around 450 known species of *Anopheles* mosquitoes, only about 60 are capable of supporting the malaria parasite's sexual development.[7] Of these, *Anopheles stephensi* (Southeast Asia), *Anopheles darlingi* and *Anopheles albimanus* (Central & South America), and the *Anopheles gambiae* species complex (sub-Saharan Africa) are among the most important in terms of maintaining disease transmission.[8] These mosquitoes are capable of supporting the sexual development of the four common human malaria-causing *Plasmodium* parasites (*Plasmodium falciparum, vivax, ovale, malariae*). *Plasmodium falciparum* is responsible for the largest burden of lethal infections in the world, and is the primary focus of vaccine and drug development.[1] Based on disease prevalence, *Plasmodium vivax* is considered to be the second most important human malaria parasite and has the unique ability (along with the geographically restricted *Plasmodium ovale*) to undergo dormancy in the liver.[58] The fifth human malaria parasite, *Plasmodium knowlesi* is primarily a zoonosis reported mainly in Southeast Asia (Malaysian Borneo) but is lethal to humans if contracted.[9] However, it is important to note it is not known if sustained human transmission of *P. knowlesi* occurs in the field; it has only been shown to be possible in an experimental setting.[10]

Regardless of the species, all human *Plasmodium* parasites are introduced into the host skin by an infected mosquito bite. The process of bloodmeal acquisition can be broken down into two distinct mutually exclusive phases: probing and imbibing.[11] During the probing phase, a mosquito salivates into host skin as it searches for blood to facilitate locating blood.[12,13] Mosquito saliva is a complex composition of molecules with various

functions, including vasodilators and anticoagulants that facilitate blood meal acquisition.[13] Since sporozoites reside in the salivary glands of the mosquito, it stands to reason that they would be inoculated while the mosquito is searching for blood (salivating into the skin). Indeed, several lines of evidence now show that the large majority of inoculated *Plasmodium* parasites are inoculated into the host skin – and not directly into circulation as was previously assumed.[14, 15, 16, 49, 57, 59] Moreover, the total number of parasites inoculated by the mosquito is quite low.[49, 63] For example, Medica & Sinnis estimate that the median number of sporozoites inoculated is eighteen.[49] Of the inoculated sporozoites, only about 20% exit the skin, leaving over several hours in a slow trickle from the site of inoculation.[18, 35] Yamauchi et al. show using the rodent parasite *Plasmodium yoelii* that most of the parasites remain in the dermis up to at least an hour post-inoculation.[35] This gradual exit from the site of inoculation suggests the possibility of previously unappreciated interactions between *Plasmodium* parasites and the host immune system. Importantly, given the time the parasite spends in the dermis, Yamauchi et al reinforce the possibility that neutralizing antibodies specific to the parasite could play an important role in the host immune response against *Plasmodium* infection.[35]

Once in the skin, the parasite must find and invade blood vessels in order to reach the liver, where it then establishes infection.[16] The parasite moves in the skin using a substrate-dependent mechanism of motility called gliding motility.[17] From the point of inoculation, the sporozoites initially move outwardly in relatively linear trajectories. After some time, there is a noticeable change in sporozoite motility patterns, with their movement becoming more circular.[18] This constrained movement is particularly seen when sporozoites are in the vicinity of blood vessels, suggesting that sporozoites are able

to sense their environment and alter their movement accordingly.[18] The precise signals, if any, that regulate these motility patterns are not yet known. In addition, the mechanism of blood vessel invasion by sporozoites is also an unanswered question, but a recent study suggests that sporozoites might display a tropism for blood vessels with curvatures similar to their own.[19]

Following the invasion of a blood vessel, *Plasmodium* sporozoites reach the liver within minutes and invade hepatocytes, crossing the liver sinusoids through either Kuppfer or endothelial cells.[20, 21] After invasion, sporozoites form a parasitophorous vacuole and begin to differentiate and divide to give rise to tens of thousands of merozoites. The infected hepatocyte ruptures, and merozoites are released into the circulation where they invade red blood cells.[22, 23] The erythrocytic stage of malaria infection is responsible for the clinical symptoms that are generally associated with malaria. These symptoms are commonly characterized by recurrent fevers, chills, headache, and nausea. It is important to note that in contrast to the symptomatic erythrocytic stage, the pre-erythrocytic stage has no symptoms associated with it, and is clinically silent.

During the erythrocytic stage, some of the parasites differentiate into male and female gametocytes, which are taken up by a mosquito, differentiate into gametes which fuse to form an invasive ookinete which traverses the mosquito midgut epithelium, eventually forming an oocyst on the basal side of the mosquito midgut epithelium. It is within the oocyst that sporozoite development occurs. Upon rupture of the oocyst, sporozoites are released into the hemocoel and invade the salivary glands of the mosquito, ready to be inoculated into the host at the next feeding.

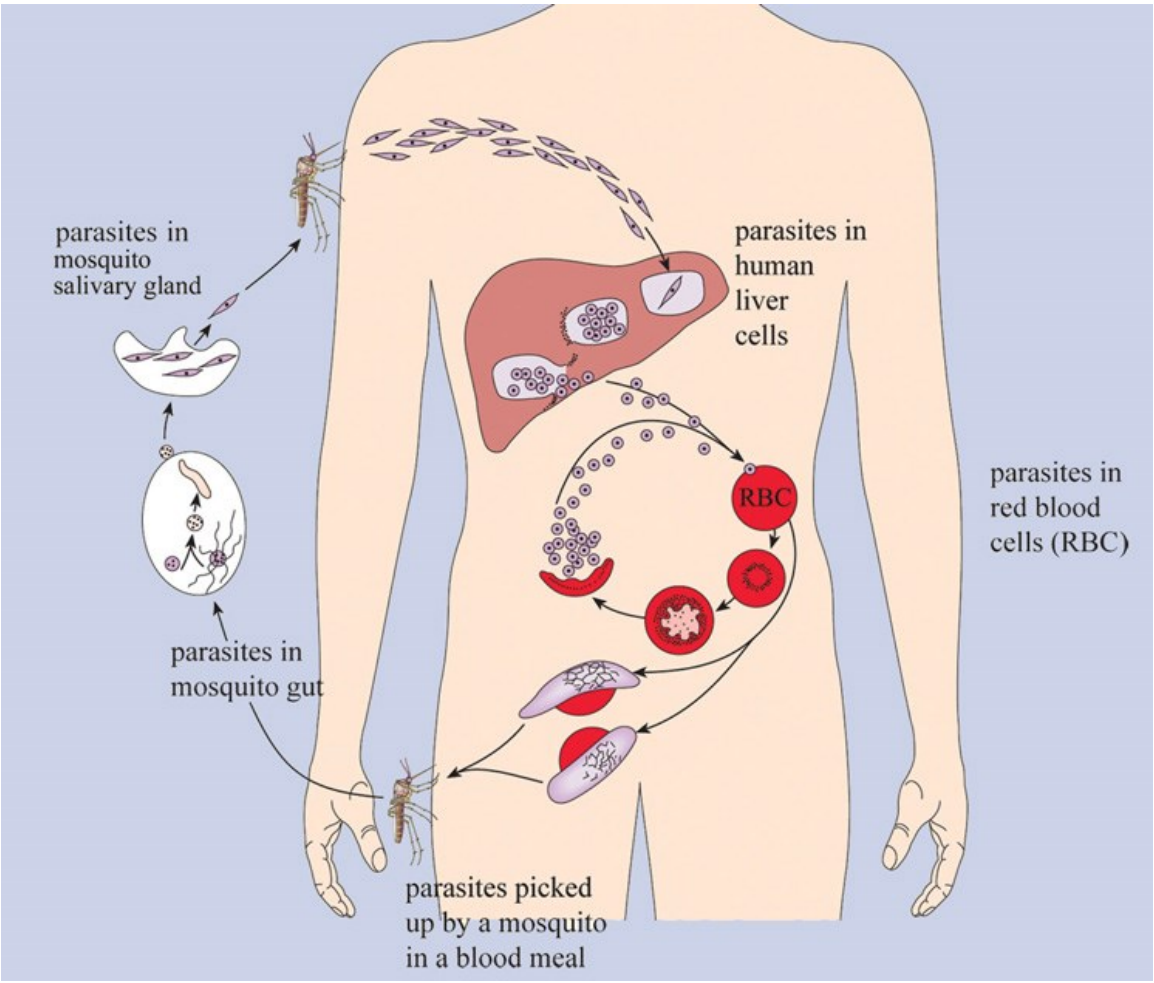


Figure 2. The lifecycle of human *Plasmodium* parasites. The illustration highlights the three stages of development within the host (pre-erythrocytic stage, asexual erythrocytic stage, and intra-erythrocytic gametocyte stage), as well as the sexual stage in the mosquito vector.

Image credit: Creative Commons Media.

In the *Plasmodium* parasite's life cycle in the host, there are two bottlenecks that can potentially be exploited for vaccine development (Figure 3).[5, 24] These are the two transmission stages of the parasite, sporozoites and gametocytes. These bottlenecks have been the basis of pre-erythrocytic and transmission blocking vaccines.[5] It is important to note however that whereas transmission blocking vaccines aim to reduce disease transmission through the mosquito, only pre-erythrocytic stage vaccines aim to prevent the development of malaria infection and symptomatic disease. Because of this, the pre-erythrocytic stage was previously identified as a vaccine target, which led to the development of RTS,S – the only pre-erythrocytic malaria vaccine under consideration for licensure.[25]

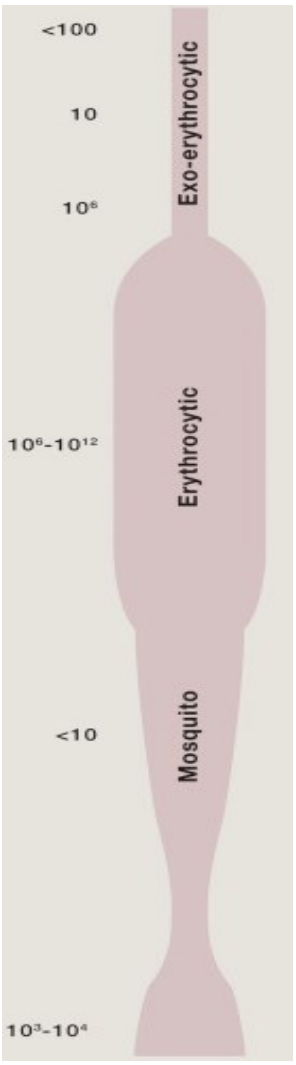


Figure 3. Schematic of *Plasmodium* parasite bottlenecks. Bottlenecks are points in the parasite lifecycle at which it has low numbers in the host and is, in theory, a vulnerable target. These bottlenecks may be exploited for malaria vaccine development.

Image credit: Modified from Cowman, A.F., Healer, J., Marapana, D. and Marsh, K., 2016. Malaria: Biology and Disease. *Cell*, 167(3), pp.610-624.

Malaria Vaccine Development & RTS,S – humanity's first malaria vaccine

Malaria vaccine development has been a work in progress since the 1960s when radiation-attenuated sporozoites (delivered by 1000 mosquito bites) were shown to induce sterile protection in 100% of the volunteers when re-challenged with infected mosquitoes.[26] In addition to the logistical challenges inherent in delivering a thousand infected bites with irradiated mosquitoes, there are additional regulatory hurdles that need to be considered. Hence, vaccination by irradiated mosquito bites is an unlikely vaccination approach for malaria. However, a number of alternative vaccine strategies have now been tried, including radiation attenuated whole sporozoites, genetically attenuated whole sporozoites, DNA-based approaches, as well as virally vectored strategies.[27] The most successful candidate to date has been the subunit vaccine candidate called RTS,S, which received a positive scientific opinion by the European Medicines Agency in 2015.[28]

RTS,S, first developed in 1987 as a collaboration between Glaxosmithkline (GSK) and the Walter Reed Army Institute of Research, contains part of the central repeat region as well as a CD4+ T-cell epitope of the major surface sporozoite antigen called the circumsporozoite protein (CSP) recombinantly expressed in yeast.[29] This is then fused to the hepatitis B surface antigen as a carrier (Figure 4). Moreover, the vaccine is delivered with AS01E, a unique liposome-based adjuvant system that elicits strong humoral and cellular immunity.[30]

Unfortunately, results from the recently completed Phase III clinical trials paint a somewhat disappointing picture. The vaccine was administered to 6-12 week olds as well as 5-17 month olds at 0, 1, and 2 months, with a booster dose at 18 months. Results of the Phase III trials show a protective efficacy of 25.9% in the 6-12 week cohort and 36.3% in the older cohort.[31] In addition to the low degree of protection RTS,S elicits, the induced protection decreases over a year.[31] These results fall short of meeting the 2015 goals of the Malaria Vaccine Technology Roadmap, according to which an effective malaria vaccine must provide at least 50% protection against death and severe disease for a year, and prevent at least 75% of clinical cases.[32]

However, interestingly, post-trial analyses of RTS,S have provided some key insights. These analyses show that in the 5-17 month cohort, RTS,S appears to have been more protective against malaria infection from parasites with genotypes that match the CSP allele on which RTS,S is based.[33] Although in the phase III trial, these matched parasite strains amounted to only 10% of the total parasite population, the protective efficacy against this strain for the 5-17 month cohort was 50.3% following vaccination (compared to 33.4% against the mismatched strains).[33] Another key observation in the post-trial analysis of RTS,S has been the strong association between protection against infection and anti-CSP antibody titers.[34] Anti-CSP antibody titers were found to be higher in the 5-17 month cohort (compared to the 6-12 week cohort), and could predict protection against clinical malaria effectively, suggesting that anti-CSP antibody titers can be used as an immune surrogate of protection against malaria.[34] It is worth noting that anti-CSP antibodies are one part of immunity against clinical disease, which also includes strong CD4+ and CD8+ T-cell responses.

Given the importance of antibodies targeting CSP, as the RTS,S post-trial analyses show, and the time the malaria parasite spends in the dermis prior to subsequent liver infection, it is surprising how little is known regarding the potential interactions between host antibodies and *Plasmodium* parasites in the skin. It is particularly important to study these interactions in the context of mosquito-based transmission of *Plasmodium*, since components of mosquito saliva may interact with the parasite to mediate the pathogenesis of disease as well as host immunity. Understanding parasite-antibody interactions in the skin could significantly contribute to an enhancement of the current vaccine effort, as we pursue the development of second generation malaria vaccines, improving the protective efficacy of RTS,S, which has been shown to partly rely on anti-CSP antibodies. However, no robust method for studying the impact of antibodies in the skin against the malaria parasite has previously been developed.

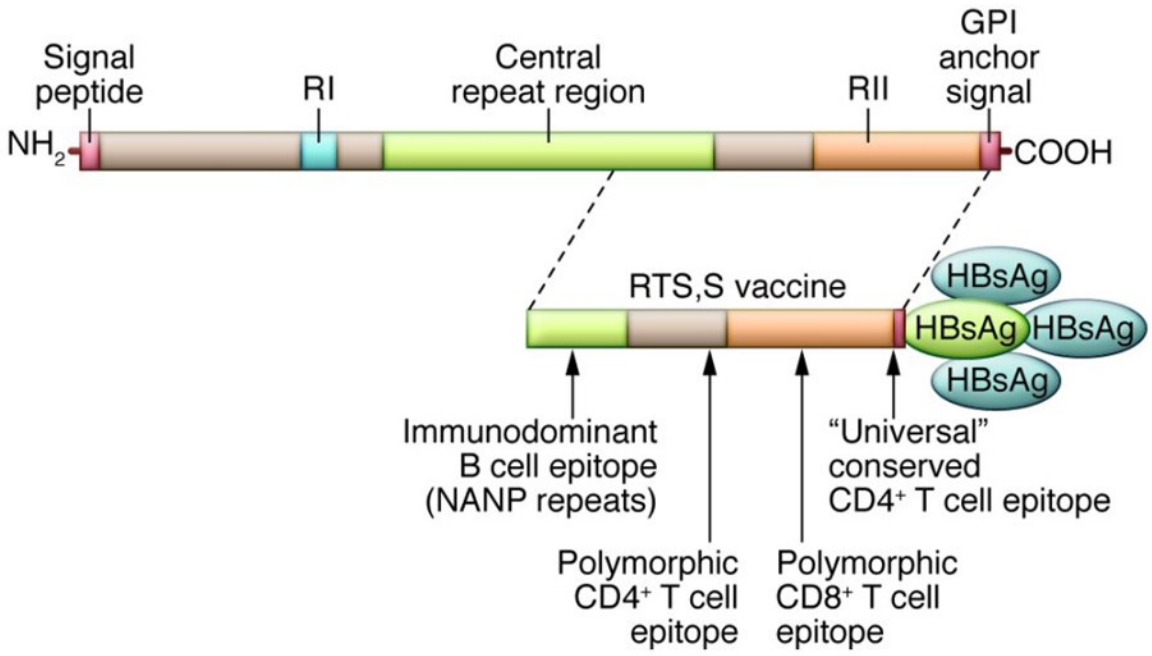


Figure 4. A schematic of CSP and RTS,S vaccine. The vaccine contains part of the central repeat region of CSP, the most abundant surface antigen on the sporozoite surface, fused to the hepatitis B surface antigen.

Image credit: Crompton, Peter D., Susan K. Pierce, and Louis H. Miller. "Advances and challenges in malaria vaccine development." *The Journal of Clinical Investigation* 120.12 (2010): 4168-4178.

Established Methods for Malaria Parasite Inoculation

Many of the original studies, performed in birds, rodents, and humans for studying malaria pathogenesis as well as vaccine development, were done using infected mosquito bites as the route of parasite transmission. However, a key limitation of using mosquitoes for inoculating parasites is that it is difficult to precisely quantitate and/or predict the dose of the inoculum.[35, 36] Given these challenges, more predictable methods for parasite inoculation were explored. One of the earliest comparisons of the different routes of parasite inoculation were done with *Plasmodium gallinaceum* using *Aedes* mosquitoes to infect chicks.[37] These included intravenous, intrahepatic, intramuscular, subcutaneous, oral, and intraperitoneal routes of parasite inoculation. Of these, intravenous inoculation of parasites was found to be most efficient at reliably resulting in blood-stage malaria infection.[37]

Following the discovery of the rodent malaria parasite, *Plasmodium berghei*, intravenous methods of inoculation were regularly performed for immunizing and subsequently challenging rodent models with *P. berghei* sporozoites for studying host immunity against the pre-erythrocytic stages of malaria. However, at the time, it was supposed that mosquito inoculation of parasites mimicked intravenous inoculation of parasites, and the skin stage of malaria infection was largely ignored. Given the growing appreciation that most of the sporozoites inoculated by mosquito bite in mosquito saliva are inoculated into the skin and can spend several hours there, alternate routes of parasite inoculation have since been explored.[35]

A more biologically-relevant route of parasite inoculation is the intradermal route, with the key advantage that it attempts to mimic potential host-parasite interactions in the dermis.[38] This involves dissecting mosquitoes, quantifying sporozoites, and injecting them in solution into the dermis of the experimental animal. Although this approach is much better than intravenous inoculation of parasites for studying the pre-erythrocytic stage of malaria infection, mechanical injections can be challenging to reproduce. This is in part due to the manual dexterity that is required from the experimenter. In addition, there is a growing body of evidence suggesting that complex interactions between components of mosquito saliva and a pathogen may enhance disease pathogenesis.[39, 40, 41, 42, 43] Consequently, there has been an increasing appreciation of parasite inoculation by mosquito, and a more standardized method of studying host immunity against the pre-erythrocytic stage is needed.

Using the rodent malaria model, *Plasmodium berghei*, the following work will establish a mosquito bite challenge model (normalized to the liver parasite burden) as an alternative to intradermal methods of parasite inoculation for studying the pre-erythrocytic stage of the malaria parasite. We then use this model to compare the protective efficacy of two different doses of an antibody targeting *P. berghei* sporozoites using intravenous and mosquito bite routes of parasite inoculation. Specifically, we test the hypothesis that antibodies targeting the malaria parasite have greater efficacy against parasites delivered via mosquito bite due to the fact that the parasite is extracellular for the longest period in the skin. We anticipate this work will have implications for future laboratory studies of malaria vaccine candidates in rodent models.

METHODS

Source of Animals

Female C57BL/6J mice were purchased from Taconic Farms (Derwood, MD), and housed in the animal facility at the Johns Hopkins Bloomberg School of Public Health. The age of the mice ranged from 5-8 weeks. The animal work presented in this thesis was done in accordance with the Animal Care and Use Committee (ACUC) guidelines (Protocol #M014H363). The Johns Hopkins University ACUC is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Methods for determining infectivity of mosquitoes

Anopheles stephensi mosquitoes were reared in the insectary at the Johns Hopkins Bloomberg School of Public Health (Baltimore, MD). Prevalence of infection in the cage was determined on Day 18-21 post-infectious blood meal by microscopically examining the salivary glands from at least 20 female mosquitoes for the presence of sporozoites after applying manual pressure to rupture the gland.

To determine the average sporozoite load in the mosquitoes, at least 10 *Anopheles stephensi* females were dissected in ice-cold Leibovitz's L-15 medium (#11415064, Thermo Fisher Scientific) Dissected glands were transferred to a pre-chilled 1.5 mL Axygen Maximum Recovery tube (#MCT-175-L-C, Axygen Scientific) with 150 µL L-15 medium. The glands were centrifuged (4°C) for a few seconds, homogenized manually using a plastic tissue grinder (#PES15BSI, Axygen Scientific), and left on ice

for 5 minutes. A 1:10 dilution was prepared from the sporozoite solution, and the sporozoites counted using a hemocytometer. For mosquito bite experiments, mosquitoes were only used if the average sporozoite load per mosquito was greater than 10,000 sporozoites and the mosquito cage had a prevalence of infection of 90% or greater.

Mosquito bite challenge experiments

Female mosquitoes were aspirated by mouth in batches of 25-30 mosquitoes to prevent potential mechanical damage caused by overcrowding in the aspirator tube. The mosquitoes were anesthetized on ice and gently transferred to a pre-chilled glass petri dish. Using forceps that were padded at the tip with tape (to prevent mechanical damage), the mosquitoes were lifted by the leg and transferred to plastic tubes (50 mL Falcon, Corning Life Sciences) with a screw cap at one end and net at the other. No more than four mosquitoes were placed in a single tube. The mosquito containers were then placed in the growth chamber (net-side up) and supplied with water to prevent death by dehydration. Around 6h prior to mosquito bite challenge, the water was removed.

Female C57BL/6J mice were anesthetized with intraperitoneal injection of ketamine (35-100 μg/g body weight) and xylazine-hydrochloride (6-15 μg/g body weight) and placed on a warming plate set to 37°C. This was done to prevent a drop in body temperature of the mice to facilitate mosquito bites. After gently blowing on the mosquitoes (to activate their host-seeking response by carbon dioxide), the containers were gently pushed up against the mouse ear (Figure 5). A timer was set to three minutes and the mosquitoes in the container were observed carefully. Once mosquitoes land on the ear, they probe a variable number of times before initiating blood feeding (personal

observation). Following observation of the desired number of mosquito bites, the mice were labeled and gently placed into a new mouse cage. Unless otherwise indicated, each ear was exposed to 4-5 mosquito bites.

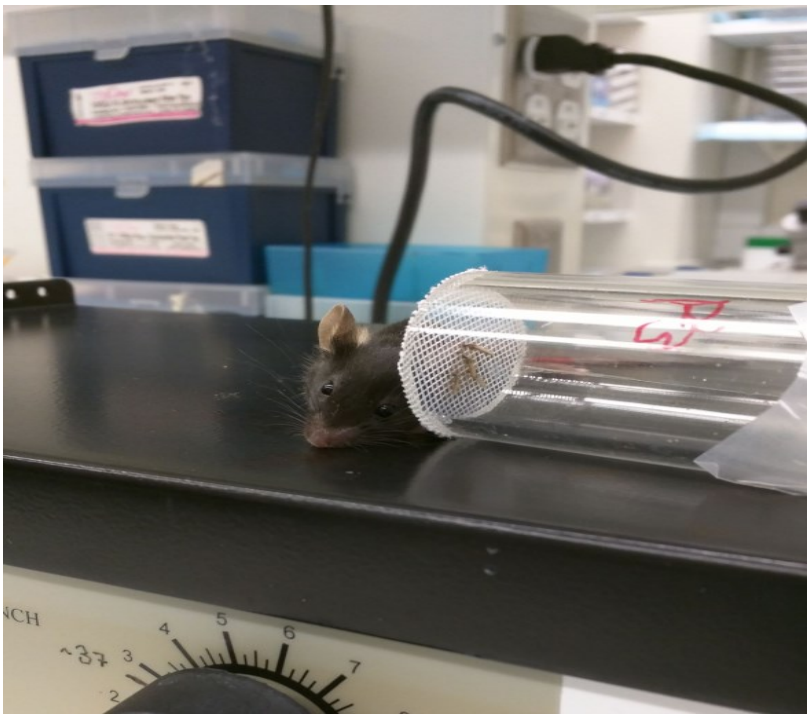


Figure 5. The mosquito bite challenge set-up. Four female *Anopheles stephensi* mosquitoes in a tube can be seen feeding on the ear of a C57BL/6J female (anesthetized with K/X and its body temperature regulated using a heating block set to 37°C). Each container of mosquitoes was allowed 3 minutes, and the number of mosquito bites within that time period observed. The total number of mosquito bites were distributed over both ears, with an approximately equal number of mosquito bites allowed on each ear.

RNA Extraction & cDNA Synthesis

40 hours after parasite challenge, mice were anesthetized with 150 µL K/X and sacrificed using cervical dislocation. The livers were dissected, washed twice in cold PBS (1X, pH 7.4), dried over kimwipes, weighed, and homogenized in 10 mL of Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH), using a manual homogenizer (Kinematica, Bohemia, NY) for 1 minute at full speed. The homogenates were allowed to sit at room temperature for 30 minutes and then 1 mL of the homogenate added to 200 µL chloroform. Following vortexing for 15 seconds, the samples were allowed to sit at RT for 15 min, and centrifuged for 15 minutes at 12,000 x g (4°C). 450 µL of the aqueous phase was added to an equal volume of Isopropanol and vortexed for 10s. After a 10 minute incubation at room temperature, the samples were centrifuged at 15,000 x g for 10 minutes. The pellet was washed with 70% ethanol, air dried for 5 minutes, and incubated in 200 µL of DEPC-treated water at 43°C for 1 hour. Standard RNA solutions (1µg/µL) were prepared and stored at -80°C.

Reverse transcription was done using 1.5 µg total RNA and random hexamers as according to manufacturer's instructions. The cycling profile was 25°C (10 min), 42°C (20 min), 95°C (5 min), and 5°C (5 min).

Quantification of Parasite Liver Burden by RT-qPCR

The protocol for quantifying *P. berghei* 18s rRNA in mouse liver is well-established and is a modification of the protocol described first by Bruña-Romero et al.[44]

Primers used for amplifying the *P. berghei* 18s rRNA gene were first described by Kumar et al.[45] and the same ones were used here:

Pb-18S-rRNA-5' GGAGATTGGTTTTGACGTTTATGTG

Pb-18S-rRNA-3' AAGCATTAAATAAAGCGAATACATCCTTA

P. berghei 18s rRNA copy numbers were calculated based on a standard curve prepared using 10-fold serial dilutions (10^7, 10^6, 10^5, 10^4, 10^3, and 10^2) of the *P. berghei* 18s rRNA gene.

Antibody Clearance: Serum Collection & ELISA

50 µg mAb 3D11 was intravenously administered to 8 week old female mice. Approximately 50 µL of blood were collected by retro-orbital plexus under anesthesia (90 mg/kg ketamine and 10 mg/kg xylazine) at 6h, 12h, 18h, 24h, 48h, 72h, and 96h following antibody inoculation. Serum was isolated by centrifuging each blood sample at 2000 x g for 10 minutes (4°C), and the supernatant saved. Serum samples were stored at -20°C.

The serum antibody levels at each point were measured by ELISA. Briefly, 96-well plates (ThermoFisher Scientific, #3455) were coated overnight (4°C) with 1.0 µg/mL of synthetic *P. berghei* CSP repeat peptide. The following day, the wells were washed 3x with Wash Buffer (0.05% Tween 20/PBS) and blocked with Blocking Buffer (2% BSA/0.05% Tween 20/PBS) for 1 hour at 37°C. Serial dilutions (1:50, 1:100, 1:200, 1:400, 1:800, 1:1600) were prepared for each serum sample in Primary Antibody Dilution

Buffer (2% BSA/0.05% Tween 20/PBS). Following a 3x wash, the wells were incubated with primary antibody for 1 hour at 37°C. The wells were washed again 3x and incubated with goat anti-mouse HRP-conjugated secondary antibody (KPL, #074-1806) diluted 1:2000 in Secondary Antibody Dilution Buffer (2% BSA/0.05% Tween 20/PBS) for 1 hour at 37°C. Following a 3x wash with Wash Buffer and 2x wash with PBS (1X, pH 7.4), the plates were developed using a commercially available kit (KPL, #50-62-00) for 5 min and the absorbance measured at 405 nm.

Immunofluorescence Assay (IFA)

In order to characterize functionality of the purified antibody, an immunofluorescent assay was performed. Briefly, 10 female *Anopheles stephensi* mosquitoes were dissected 20 days post-infectious bloodmeal and the isolated sporozoites quantified. Wells on a Teflon/Poly-L-lysine slide (TEKDON Inc., #117-051-122) were coated with 10,000 sporozoites per well and left overnight at RT to air-dry. The following day, the wells were incubated with 4% PFA (Electron Microscopy Sciences, #15710) for 1 hour at RT to fix the sporozoites. Following a 3x wash with PBS (1X, pH 7.4), the wells were blocked with 1% BSA/PBS for 1 hour at RT. The wells were washed again 3x with PBS (1X, pH 7.4), and incubated for 1 hour at RT with serial dilutions (0.1 µg/mL, 0.01 µg/mL, and 0.001 µg/mL) of old and new mAb 3D11. Following another 3x wash with PBS (1X, pH 7.4), the wells were incubated with Rabbit anti-mouse IgG (H+L) ross-adsorbed secondary antibody (Thermo Fisher Scientific, #A-11062) for 1 hour at RT. After a final 3x wash with PBS (1X, pH 7.4), the slide was air dried for 15 minutes and

mounted with Prolong Gold antifade with DAPI (Thermo Fisher Scientific, #P36935). A cover glass was placed over the slide and left overnight to dry in the dark.

Statistical Analyses

For determining the clearance kinetics of 50 µg mAb 3D11, serial dilutions of each serum sample were prepared in order to normalize across all samples. This is done by generating a standard curve for each sample and interpolating the serum dilution that corresponds to an absorbance measurement of 1.0 (a point at which the concentration of the antibody in the serum is directly proportional to the absorbance). These interpolated values can be plotted against time to observe the change in serum antibody levels over time.

For the comparison of liver parasite burden between groups, non-parametric Mann-Whitney tests were used (two-tailed, $\alpha=0.05$).

RESULTS

Comparison of Intravenous versus Mosquito Bite Inoculation of Sporozoites

Previous work in the Sinnis laboratory (Kim Wang & Photini Sinnis, unpublished) compared the efficacy of passively administered mAb 3D11 (antibody specific to *P. berghei* CSP repeats) against intravenous and intradermal routes of parasite inoculation. The results suggested that 50 µg of antibody administered approximately 24 hours prior to parasite challenge was more protective against liver infection when sporozoites were inoculated intradermally compared to intravenously inoculated parasites. Despite the greater control intradermal inoculation has afforded, it is not the natural route of pathogen transmission.

Therefore, the first aim of my thesis work was to develop a mosquito bite challenge model using *Anopheles stephensi* mosquitoes and the rodent malaria parasite *P. berghei*, controlling for the number of mosquito bites per mouse as a more biologically relevant alternative measure of exposure to malaria parasites. For the purposes of standardization between intravenous and mosquito bite inoculation of parasites, we used the parasite liver burden as measured by RT-qPCR as an endpoint. Briefly, mice were challenged either intravenously with a predetermined number of sporozoites or exposed to a predetermined number of mosquito bites. Forty hours later, the parasite burden as a function of the gene copy number of *P. berghei* 18s rRNA in the mouse liver was quantified. Since there is a scarcity of published literature comparing parasite liver burden from different doses of sporozoites inoculated by various routes, and because sporozoite infectivity may vary from one laboratory to another, we first did a dose-response curve of parasites inoculated intravenously to get a general understanding of the

relationship between liver parasite burden and sporozoite dose after IV inoculation. As shown in Figure 6, we inoculated groups of mice with 250, 2500, 5000, and 10,000 sporozoites and found a linear trend between the number of sporozoites inoculated IV and the resulting liver parasite burden.

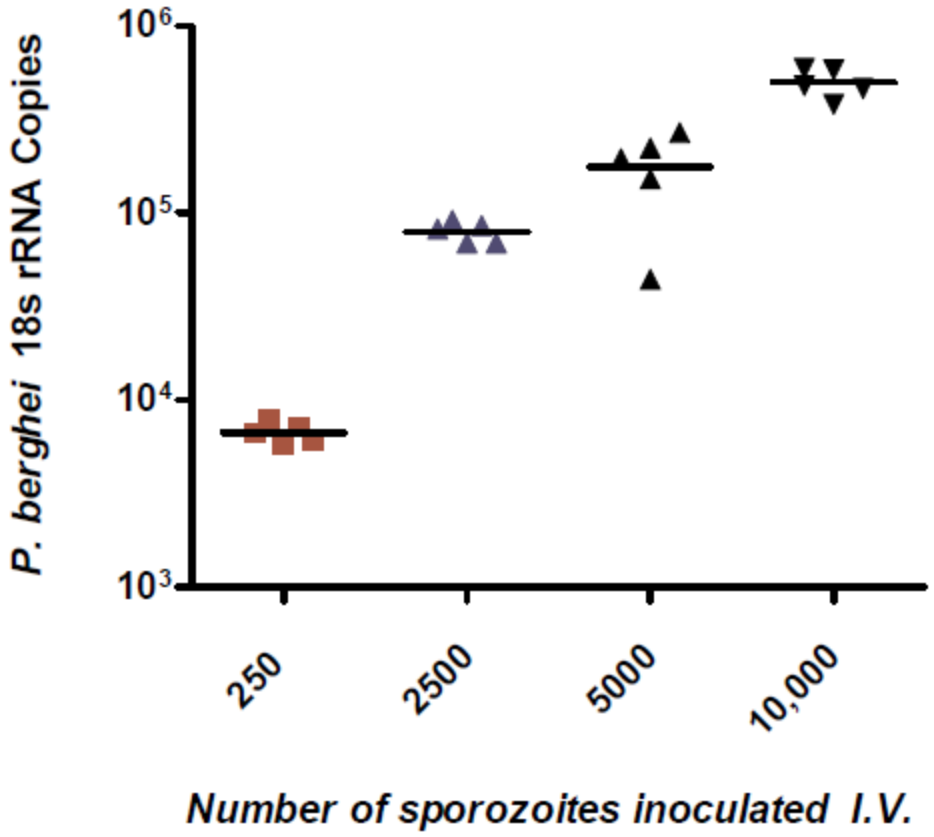


Figure 6. Liver parasite burden in groups of female C57BL/6 mice inoculated intravenously with 250, 2500, 5000, or 10,000 *P. berghei* sporozoites (n=5 mice/group). Livers were harvested 40 hrs after sporozoite inoculation, RNA extracted and *P. berghei* 18s rRNA quantified by RT-qPCR.

Similarly, in order to acquire a general understanding of the relationship between liver parasite burden and parasites inoculated by mosquito bite, we exposed groups of mice to 4, 8, or 16 mosquito bites divided over both ears (Figure 7A). In contrast to the linear trend we saw with increasing doses of IV inoculated sporozoites, we found no statistically significant difference in parasite liver burden between mice administered 8 versus 16 mosquito bites. To determine if the saturation we were observing was potentially real or a function of excessive hemorrhaging in the ear following 16 mosquito bites, we repeated the experiment and divided the 16 mosquito bites over both ears, the nose, and the tail (Figure 7B). Again, we observed the same result. Given these data, we selected 8 mosquito bites for our experiments.

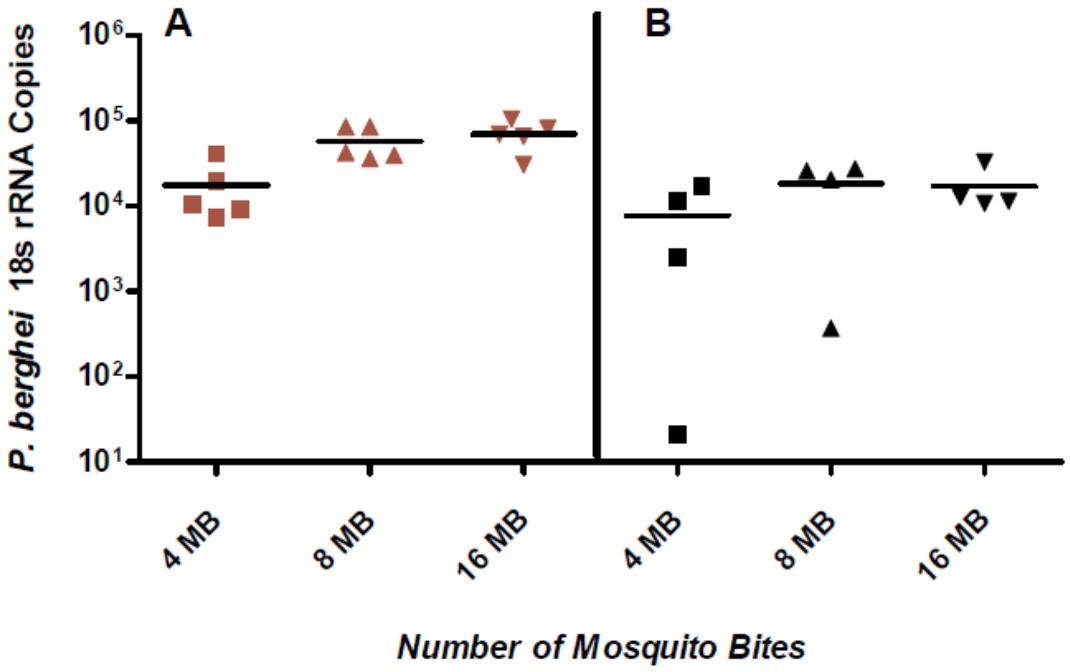


Figure 7A. Liver parasite burden in groups of female C57BL/6 mice exposed to 4 (one ear), 8 (both ears), or 16 mosquito bites (both ears) (n=5 mice/group). B. Liver parasite burden in groups of female C57BL/6 mice exposed to 4 (one ear), 8 (both ears), or 16 mosquito bites (bites divided over both ears, nose, and tail) (n=4 mice/group). Livers were harvested 40 hrs after sporozoite inoculation, RNA extracted and *P. berghei* 18s rRNA quantified by RT-qPCR.

Since the median liver parasite burden in mice exposed to 8 mosquito bites was between 10^4 and 10^5, we decided to do a second intravenous titration experiment to get better estimates of the liver parasite burden for a narrower range of sporozoites, that more closely matched the liver parasite burden for 8 mosquito bites. In this experiment, we inoculated groups of mice with 540, 900, 1500, and 2500 *P. berghei* sporozoites IV, and included a reference group that was exposed to 8 mosquito bites (Figure 8). We found that the liver parasite burden in our mosquito bite reference group most closely corresponded to the group intravenously inoculated with 540 sporozoites.

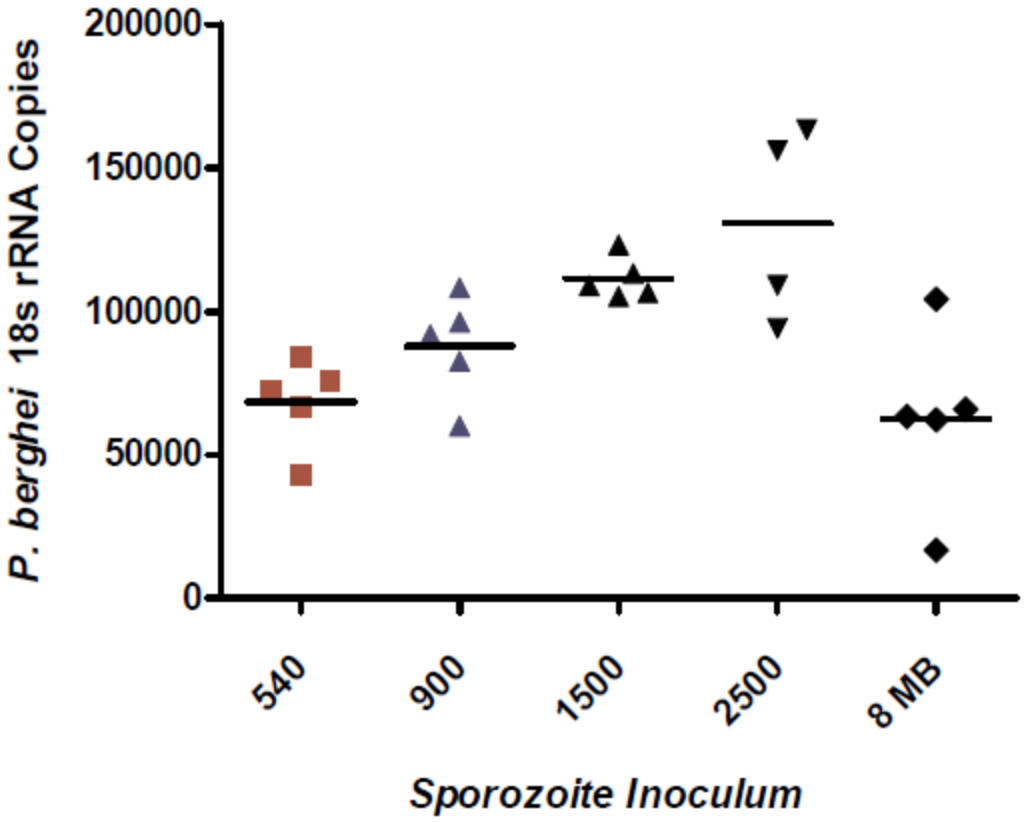


Figure 8. Liver parasite burden in groups of female C57BL/6 mice intravenously (I.V.) inoculated with 540, 900, 1500, or 2500 sporozoites. A group of mice exposed to 8 mosquito bites (M.B.) was included as reference. Mice were sacrificed 40 hours after challenge and liver parasite burden quantified by RT-qPCR. n=5 mice/group for all groups except the group receiving 2500 sporozoites I.V., where n=4 mice.

In summary, these experiments suggest that in the *P. berghei* ANKA system, 8 infected mosquito bites results in a liver parasite burden equivalent to 500 intravenously inoculated sporozoites.

Serum Clearance Kinetics of 50 μg mAb 3D11

Before we could compare the efficacy of antibodies targeting sporozoites after inoculation by mosquito bite versus intravenously, we also needed to determine the optimal time for challenging mice following antibody administration. To do this, we investigated how the level of mAb 3D11 in the serum changes over time. We inoculated four female C57BL/6 mice with 50 μg mAb 3D11, took blood samples at 6h, 12h, 18h, 24h, 48h, 72h, and 96h after mAb inoculation and determined the serum level of mAb 3D11 of each mouse by ELISA. The results shown in Figure 9 are the average of the clearance kinetics in four mice. In general, we see that the level of mAb 3D11 in the serum follows a biphasic decay, declining sharply over the first 24 hours, and then declining more stably over the next three days. After 24 hours, the serum level of mAb 3D11 was reduced by 39% compared to its level at the earliest 6h time point. Furthermore, from 24h to 48h, 48h to 72h, and 72h to 96h, the serum level of mAb declined by 15%, 11%, and 14% respectively. Based on the serum clearance kinetics, we chose to challenge mice 24 hours following antibody administration, as that was the point at which the antibody level in the serum appears to become relatively stable.

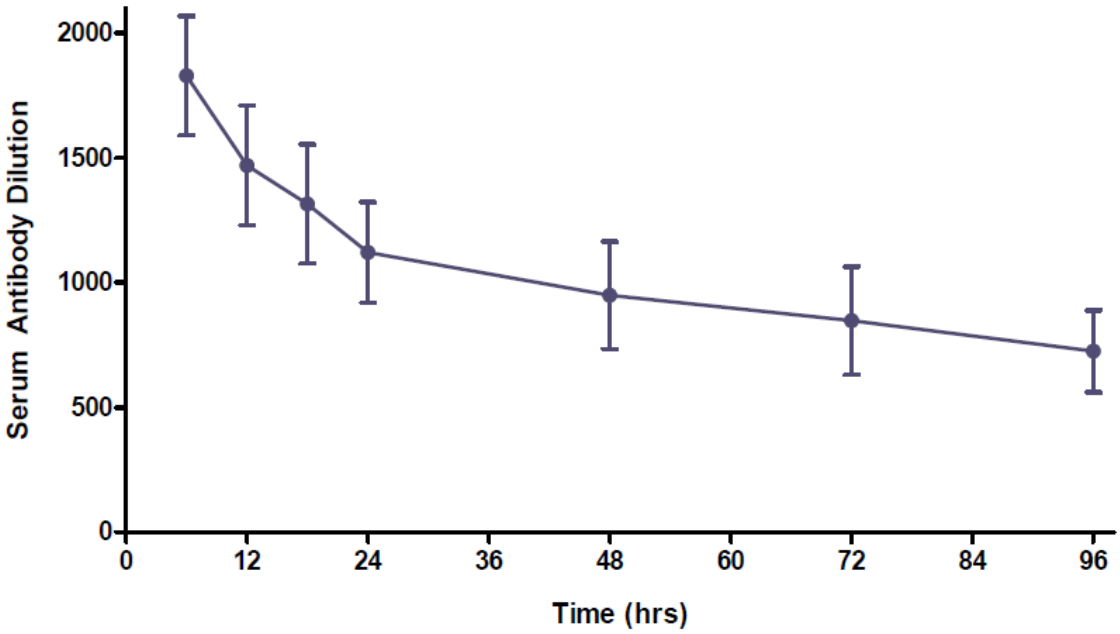


Figure 9. Average serum clearance kinetics of 50 µg mAb 3D11 in female C57BL/6 mice (n=4) over 96 hours. One serum sample from each mouse was collected at 6h, 12h, 18h, 24h, 48h, 72h, and 96h. Using ELISA, a standard curve for each serum sample was prepared by serial dilution (each dilution run in duplicate) to interpolate the serum dilution that corresponded to an absorbance measurement of 1.0 (a point at which the concentration of the antibody in the serum is directly proportional to the absorbance). Each data point shows the average (± standard deviation) of four interpolated values (one per mouse) for each time point at which sera were collected.

Protective Efficacy of mAb 3D11 After Sporozoite Challenge Delivered Intravenously versus by Mosquito Bite

Having worked out how to deliver equivalent sporozoite doses after intravenous and mosquito bite inoculation, and the optimal time for antibody administration, we created an experimental design incorporating these data (Figure 10). We then conducted a series of experiments to determine whether passively administered mAb 3D11 had the same protective efficacy against sporozoites inoculated intravenously versus by mosquito bites. We first investigated this using 50 μg of mAb 3D11 and gave the control groups an equivalent amount of mouse serum IgG, henceforth referred to as mIgG. The results for two independent experiments are shown in Figure 11A. In both experiments, we observed that 50 μg mAb 3D11 conferred less protection against intravenous challenge compared to mosquito bite challenge. However, in both experiments the liver parasite burden in our control group receiving 500 *P. berghei* sporozoites IV was statistically significantly higher than that in our control group receiving 8 mosquito bites. This can be challenging when interpreting the results, as it is not clear whether the protective effect observed in the antibody group is actually due to the antibody or if it is simply a result of fewer sporozoites reaching the liver.

Therefore, we decided to decrease the intravenous inoculum to 250 *P. berghei* sporozoites, hoping that this would allow us to achieve similar parasite liver burdens in the control groups, and repeated the experiment using 50 μg mAb 3D11 (Figure 11B). Again, we observed the same trend as before, with the antibody being more protective against mosquito bite inoculation of sporozoites. However, this time, in two biological

replicates, the parasite liver burden in our intravenous control group statistically significantly lower than that in our mosquito bite control group.

To look at our data side by side, we calculated the change in parasite 18s rRNA copy number in each treatment group compared to the average of the respective control group. In Figure 12A, these results are shown side-by-side for the four independent experiments. The difference in percent reduction between the intravenous and mosquito bite groups is statistically significant for each experiment ($p<0.001$, Mann-Whitney).

To determine the average percent reduction in the intravenous and mosquito bite groups across the four experiments, we pooled the data together (Figure 12B). In the groups receiving 50 μg mAb 3D11, compared to their respective controls, we found an average reduction in parasite liver burden of 93% in mice challenged by mosquito bite and a 69% reduction in mice challenged intravenously. These reductions, compared to the controls, were statistically significant.

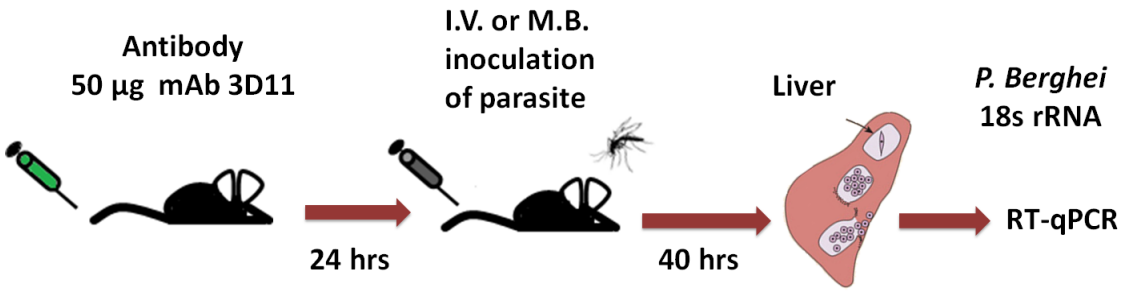
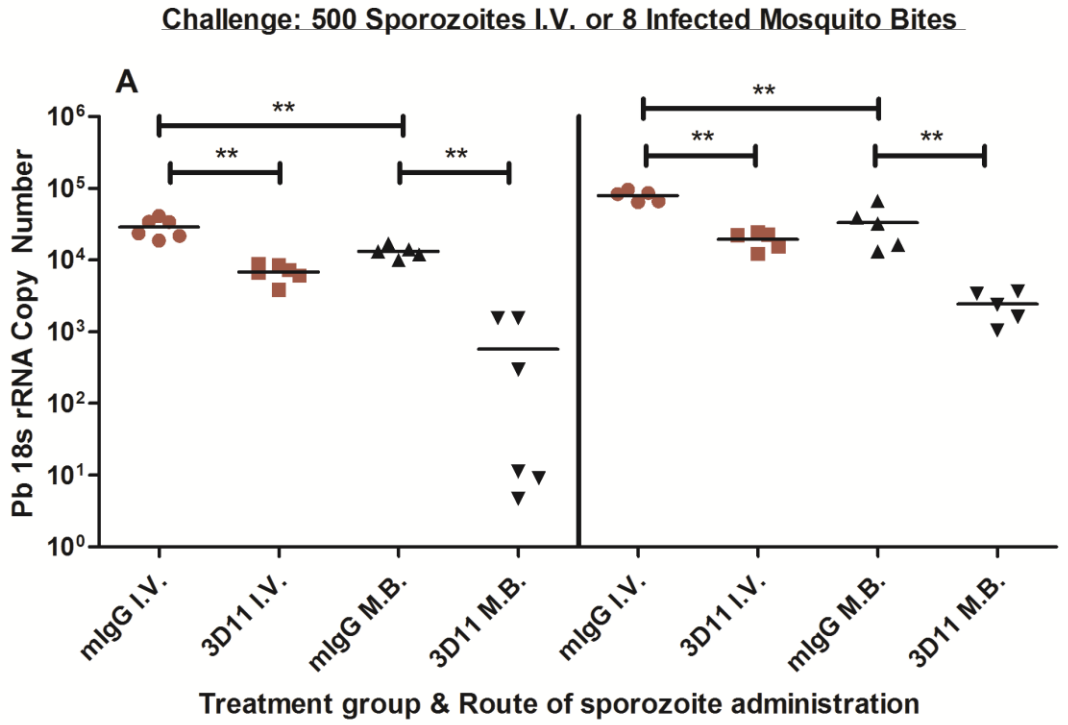
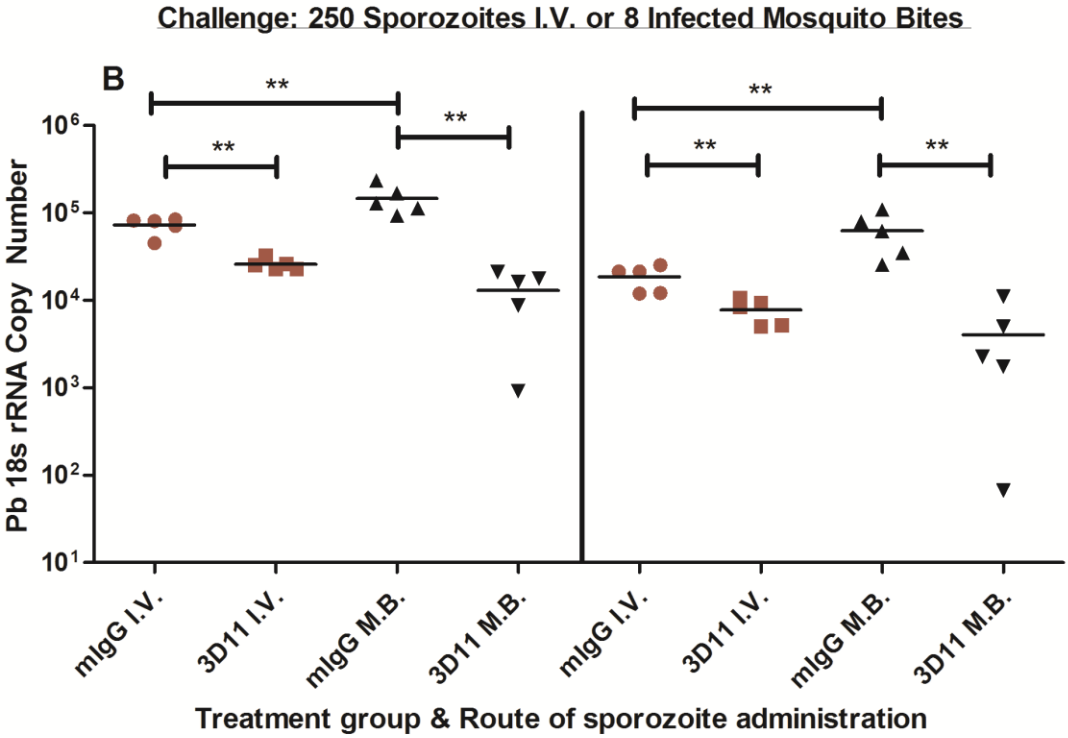


Figure 10. A schematic of our experimental design. Briefly, mice are inoculated with antibody 24h prior to *P. berghei* sporozoite challenge either intravenously (I.V.) or by mosquito bite (M.B.). 40h later, mice are sacrificed and the liver parasite burden quantified by RT-qPCR of parasite 18s rRNA.


Challenge: 500 Sporozoites I.V. or 8 Infected Mosquito Bites
A
Pb 18s rRNA Copy Number
10^6
10^5
10^4
10^3
10^2
10^1
10^0
**
**
**
**
**
**
mIgG I.V.
3D11 I.V.
mIgG M.B.
3D11 M.B.
mIgG I.V.
3D11 I.V.
mIgG M.B.
3D11 M.B.
Treatment group & Route of sporozoite administration


Challenge: 250 Sporozoites I.V. or 8 Infected Mosquito Bites
B
Pb 18s rRNA Copy Number
10^6
10^5
10^4
10^3
10^2
10^1
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mIgG I.V.
3D11 I.V.
mIgG M.B.
3D11 M.B.
mIgG I.V.
3D11 I.V.
mIgG M.B.
3D11 M.B.
Treatment group & Route of sporozoite administration

Figure 11. Liver parasite burden in mice passively immunized with 50 μg mAb 3D11 and then challenged 24h later with: A. 500 intravenously (I.V.) inoculated *P. berghei* sporozoites or 8 mosquito bites (M.B.), and B. 250 intravenously (I.V.) inoculated *P. berghei* sporozoites or 8 mosquito bites (M.B.). Mice were sacrificed 40 hours after challenge and liver parasite burden quantified by RT-qPCR. n=6 mice/group for the first experiment (top-left), and n=5 mice/group for the remaining three experiments.

** = significant, $p<0.001$, Mann-Whitney test.

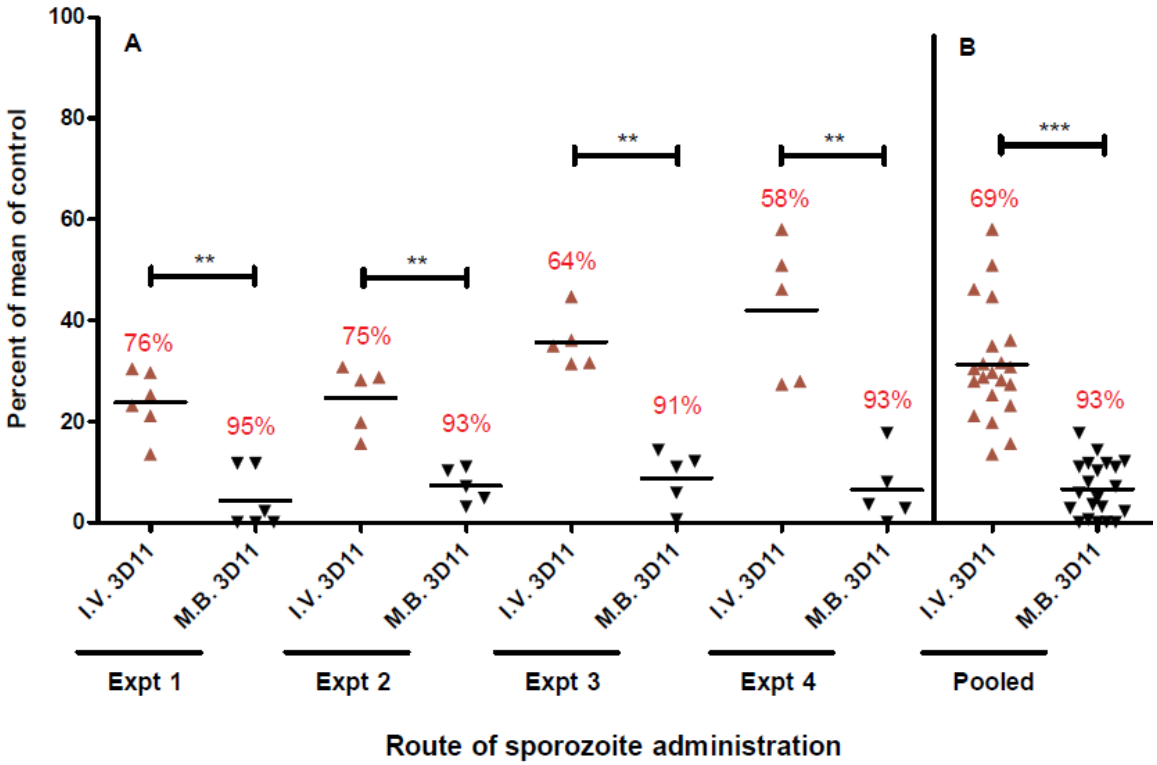

Figure 12A. Percent inhibition in liver parasite burden after passive transfer of 50 µg mAb 3D11 24h prior to challenge with sporozoites inoculated intravenously (I.V.) versus by mosquito bite (M.B.). Two replicates of two different experimental protocols are shown. Data are presented as the percent of mean of the respective controls. B. Pooled percent reduction in liver parasite burden in mice passively immunized with 50 µg mAb 3D11 against intravenous and mosquito bite challenge (4 experiments).

** = significant, $p<0.001$, *** = significant, $p<0.0001$, Mann-Whitney test.

We next wanted to determine whether we would see a similar impact on mosquito injected sporozoites with a lower dose of passively administered antibody. Given the difficulty to achieve comparable liver parasite burden in our intravenous and mosquito bite control groups, we modified the intravenous inoculum to 350 *P. berghei* sporozoites. Using 8 mosquito bites and 350 intravenously inoculated *P. berghei* sporozoites, we performed a series of experiments with 25 μg mAb 3D11 passively administered 24 hours prior to challenge. We hypothesized that we would observe a reduced level of protection in the mAb 3D11 treated groups compared to previous experiments. The results for three independent experiments are shown in Figure 13. In all three experiments, we can observe a trend consistent with what we saw for 50 μg mAb 3D11 – that the antibody is less protective against intravenous inoculation of parasites than against mosquito bite. For the first two experiments, we were able to achieve statistically similar liver parasite burden in our control groups, however, in the third experiment the intravenously inoculated control group had a liver parasite burden that was significantly higher than that in the mosquito bite control.

We then normalized the data for easier comparison by calculating the change in parasite 18s rRNA copy number in each treatment group compared to the average of the respective control group. As shown in Figure 14A the difference in the reduction between the intravenous and mosquito bite groups is statistically significant for the first two experiments ($p<0.001$, Mann-Whitney), but not significant in the third experiment. Despite this non-significance, however, the trend of greater reduction in liver parasitemia against mosquito bite holds. We also pooled the data to determine the average percent reduction in the intravenous and mosquito bite groups (Figure 14B) and found

statistically significant reductions in parasite liver burden of 89% ($p<0.001$) and 50% ($p<0.0001$) in mice challenged by mosquito bite and intravenously respectively.

Finally, in order to test the hypothesis that the protective effect we were seeing with 50 μg mAb 3D11 was dose-dependent, we compared the pooled percent reduction in the 50 μg mAb 3D11 intravenous and mosquito bite groups to the pooled percent reduction in the same groups for 25 μg mAb 3D11 (Figure 15). We saw a reduced level of protection only in the 3D11 group challenged intravenously (a change in percent reduction from 69% to 50%), and this 19% difference is statistically significant ($p<0.0001$). However, for the two antibody concentrations, we saw no statistically significant reduction in the level of protection in the 3D11 groups challenged by mosquito bite (a change in percent reduction from 93% to 89%, $p>0.05$).

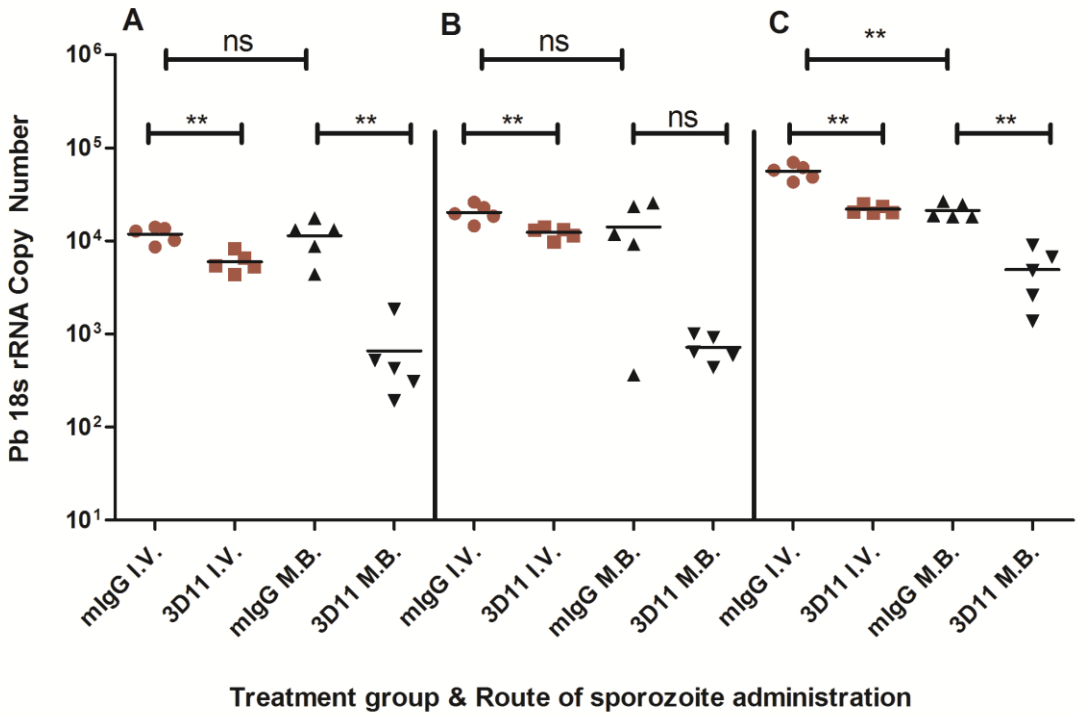

Figure 13A-C. Liver parasite burden in mice passively immunized with 25 µg mAb 3D11 and challenged 24h later with 350 intravenously (I.V.) inoculated *P. berghei* sporozoites or 8 infected mosquito bites (M.B.) (3 experiments). Mice were sacrificed 40 hours after challenge and liver parasite burden quantified by RT-qPCR. n=5 mice/group in all three experiments. ** = significant, $p<0.001$, ns = not significant, $p>0.05$, Mann-Whitney test.

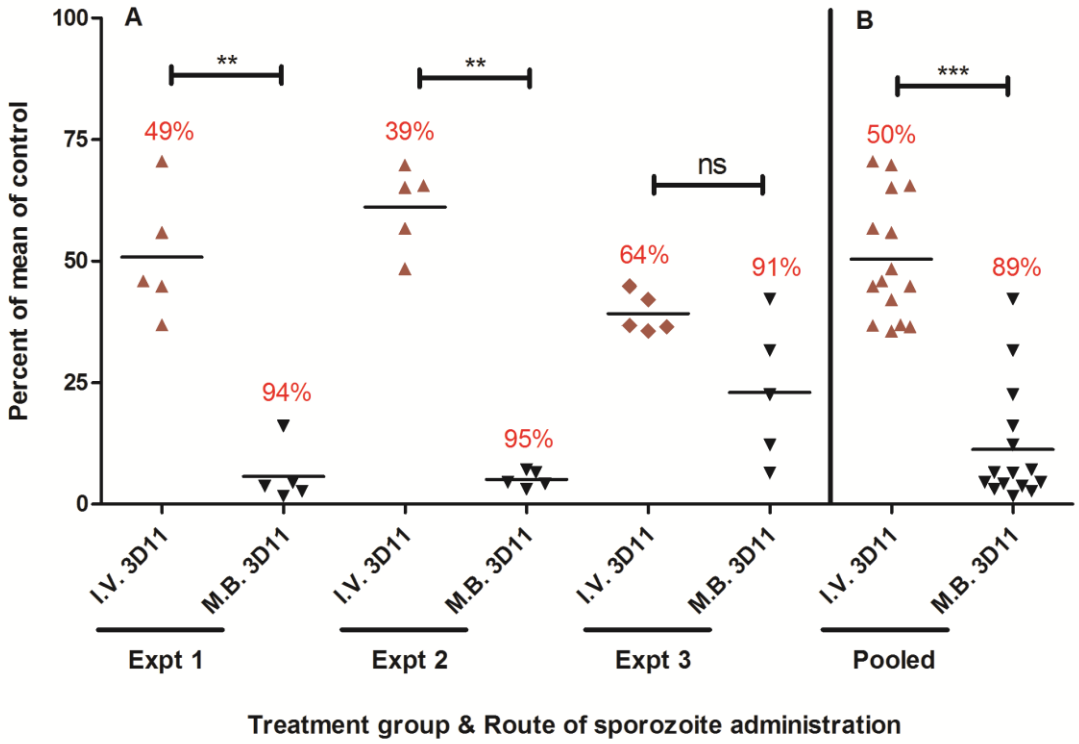

Figure 14A. Percent reduction in liver parasite burden in mice passively immunized with 25 µg mAb 3D11 and challenged 24h later either intravenously (I.V.) or by mosquito bite (M.B.) (3 experiments). Data are presented as the percent of mean of the respective controls. B. Pooled percent reduction in liver parasite burden in mice passively immunized with 25 µg mAb 3D11 and challenged 24h later either intravenously or by mosquito bite (3 experiments). ** = significant, $p<0.001$, *** = significant, $p<0.0001$, ns = not significant, $p>0.05$, Mann-Whitney test.

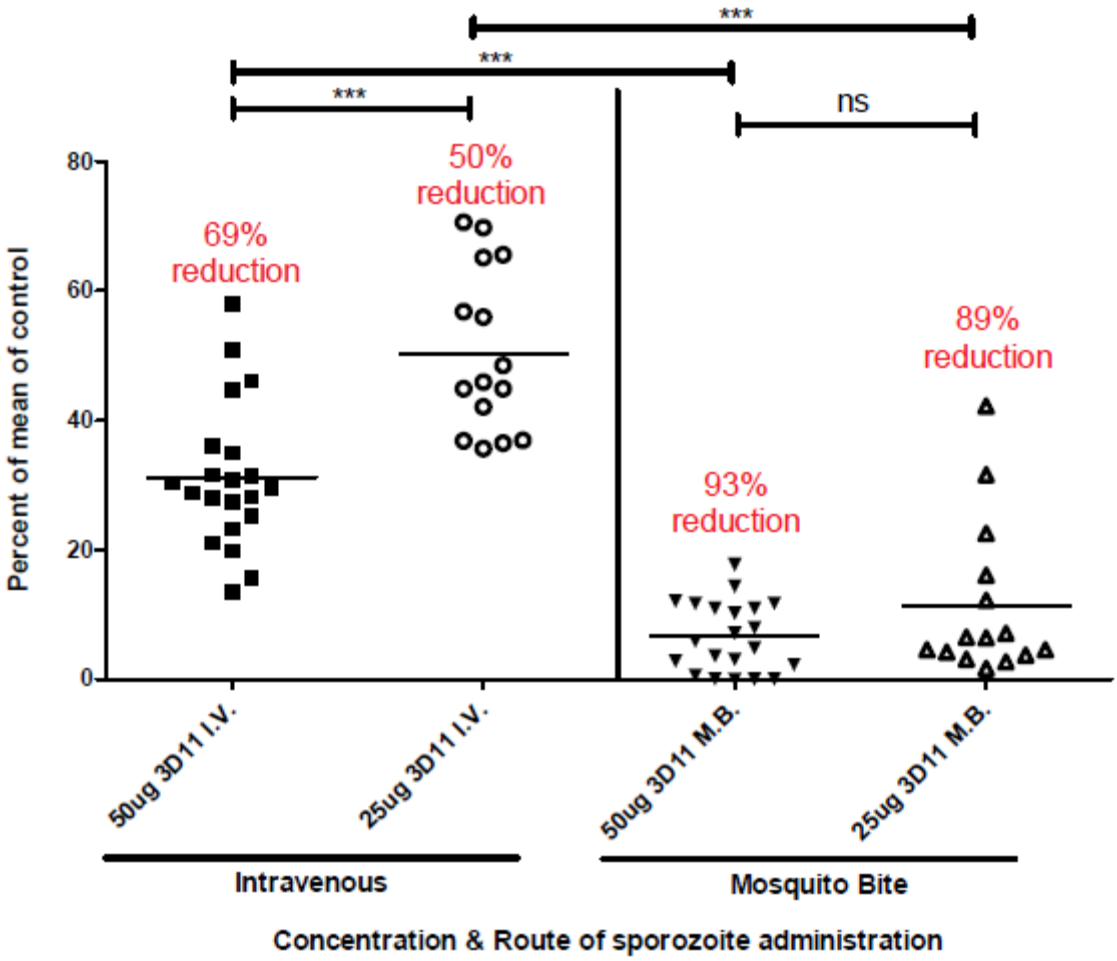

Figure 15. Comparative efficacy of two doses of mAb 3D11 (50 µg and 25 µg) passively administered to female C57BL/6 mice 24h prior to challenge by mosquito bite (M.B.) or intravenously (I.V.) inoculated *P. berghei* sporozoites. Results are shown as the percent of mean of the respective controls, and were pooled from four independent experiments for 50 µg mAb 3D11, and three independent experiments for 25 µg mAb 3D11. The relative percent reduction in liver parasite burden is shown in red. *** = significant, $p<0.0001$, ns = not significant, ($p>0.05$), Mann-Whitney test.

DISCUSSION

A Novel Method for Studying the Pre-erythrocytic Stages:

Using the rodent malaria model *P. berghei* ANKA and the C57BL/6 strain of mice we show that 350 sporozoites inoculated intravenously and 8 infected mosquito bites result in similar liver parasite burdens. Mosquito bite is the natural route of malaria infection in the field, and there has been a growing appreciation regarding the complex interplay among innate immunity in the skin, mosquito salivary gland components and the malaria parasite. However, a key reason many investigators avoid using mosquito bite as the route of parasite inoculation is the inability to precisely estimate the number of sporozoites inoculated. Assuming that the liver parasite burden is directly related to the number of sporozoites that successfully reach and invade hepatocytes, we established a method to standardize the route of infection using parasite liver burden as our endpoint measure. The significance of this approach is that it allows for a separation of the impact of an intervention, such as passively transferred antibodies, on sporozoites in the circulation versus sporozoites in both the circulation and the skin. Hence, this methodology provides further insight into the role of the skin phase during the initial stages of infection.

It is worth noting that a key limitation of using our model, while indispensable for studying host immunity against the pre-erythrocytic stage, is that it is time and resource intensive. Moreover, we note that there can be potential differences from one laboratory to another in the quality of mosquito infection, viability of dissected sporozoites, strain of mice, and strain of *P. berghei* used.[60] Therefore, we recommend that the method we have established in this work (350 sporozoites versus 8 mosquito bites) be used as a starting

point for optimizing the model in other laboratories. In addition, our methodology may be extended to compare the efficacy of treatments against intradermal versus mosquito bite inoculation of parasites. Such experiments could potentially provide further insight into the potential role of mosquito saliva in modulating host immune responses.

Interestingly, we found that the liver parasite burden saturates beyond 8 mosquito bites (Figure 7A-B). A recent paper by Liehl et al. shows the role of a type one interferon (IFN) response in hepatocytes triggered by *Plasmodium* RNA.[57] We speculate that this could be a parasite-induced phenomenon to either prevent overwhelming the host or prevent priming of a strong CD8+ T-cell response (which is an important component of host immunity against the liver stages).[59, 63] However, it is interesting to note that we don't observe a similar saturation in liver parasite burden in response to increasing numbers of intravenously inoculated sporozoites. One reason for this discrepancy could be that the effectiveness of the hepatocyte innate immune response is dependent on gradually increasing numbers of hepatocyte infections. This is in line with work by Yamauchi et al. showing that sporozoites leave the dermis in a gradual manner.[35] It is possible that the IFN-dependent innate immune response is overwhelmed by the sudden influx of large numbers of intravenously inoculated sporozoites. Nevertheless, we think this could be an interesting question to address in future work.

Can antibodies act in the skin?

The skin is the largest organ of the body and our first defensive barrier against many pathogens. Pathogens that are able to make it past the epidermis end up in the

dermal layer. It is within this compartment that malaria-causing *Plasmodium sp.* are inoculated by the mosquito. In the past decade or so, there has been a growing appreciation of immunity in the skin. Orchestrating this immune response are a diverse array of lymphoid cells including resident macrophages (also called Langerhans cells), mast cells, resident CD4+ and CD8+ T cells, dendritic cells, innate lymphoid cells, and B cells.[50, 51] In the context of malaria infection and immunity, the dermis is the most important layer in the skin. This is because mosquitoes inoculate the majority of their parasite inoculum into the dermis.[14, 15, 16, 49] What is important to note is that of the total number of parasites inoculated by the mosquito (a highly variable number, with an average of 123 sporozoites but median of 18), only about 20% of the sporozoites exit the skin.[18, 49] Moreover, sporozoites can take up to two hours to exit the skin, leaving in a slow trickle from the site of inoculation.[35] Therefore, from the perspective of vaccine immunology, targeting the sporozoites in the dermis, where they are most vulnerable for the longest period of time promises to be an effective approach for future malaria vaccines.

Using intravital microscopy, Vanderberg showed that inoculating mice with 320 µg of mAb 3D11 led to the complete immobilization of sporozoites inoculated by mosquito bite into a mouse ear.[47] Since 320 µg is much higher than what is observed in naturally-infected and immunized individuals, we sought to investigate this observation using our methodology using lower doses of antibody. We first compared the protective efficacy of 50 µg of mAb 3D11 against intravenous and mosquito bite inoculation of sporozoites 24 hours after antibody treatment. We selected 24 hours as the time of parasite challenge because that was the point at which antibody level in the serum

becomes relatively stable (Figure 9). We show that the same dose of antibody is significantly more protective against mosquito bite than intravenously inoculated sporozoites. These results are similar to findings from another study that compared liver infection using mAb 2F6 and rodent parasite *Plasmodium yoelii*, which show a greater inhibition in liver parasitemia (as measured by bioluminescence) against mosquito bite compared to intravenous challenge of sporozoites.[56]

Future directions

Our results emphasize the need for evaluating pre-erythrocytic vaccine candidates, specifically ones inducing a strong antibody response, using mosquito bites as the route of challenge in order to take potential sporozoite neutralization in the dermis into account. Moreover, these evaluations can be enhanced by including intravenously challenged treatment groups to determine the potential effect antibodies induced by vaccination may have on parasite inhibition in the skin. For instance, in a recent study evaluating the efficacy of a genetically-attenuated whole sporozoite vaccine,[48] the authors challenged immunized mice intravenously, and concluded that antibodies were playing a minor role in limiting liver infection. Given our results, these conclusions may have missed an important component of host immunity against pre-erythrocytic stage of infection.

The methodology we have established here may also be combined with chimeric rodent malaria parasites to allow for the *in vivo* evaluation of vaccine candidates against human *Plasmodium* parasites, for which we lack suitable animal models. For example, mice immunized using *P. berghei* parasites with CSP repeats from *P.falciparum*[61] or

P.vivax[62] could be challenged using our methodology to gain a more comprehensive picture of host immunity against human *Plasmodium* parasites *in vivo*. This could help in narrowing down promising vaccine candidates for future clinical development.

Our results of greater antibody-mediated protection against mosquito bite, suggest that mAb 3D11 might be acting within the dermis. However, if this is true, how does this antibody get there? We looked in the literature for potential candidates among known IgG receptors.[55] There are a number of proteins in the Fc family of receptors that are capable of binding IgG antibodies. However, to date, only a single receptor is known to bind as well as transport IgG antibodies across cells. This is the neonatal Fc receptor, FcRn, and was first discovered in the context of maternal immunity, in its central role in the passive transfer of maternal antibodies to the fetus.[52] In addition, FcRn is also responsible for significantly extending the half-life of IgG antibodies in the serum.[52] Research on FcRn over the past few decades has primarily focused on its exploitation for extending the half-life of drugs by engineering drugs with an Fc region that is specific to therapeutic IgG antibodies.[54] However, in addition to its expression in syncytiotrophoblasts, FcRn is also expressed in the lungs, kidneys, and in endothelial vessels.[53] But no one has yet explored the potential role of FcRn in transporting IgG antibodies from the vasculature into the dermis. An interesting future avenue to pursue could be to investigate whether passively administered mAb 3D11 is as protective against mosquito bite in FcRn -/- mice, as we observe in wild type mice.

In conclusion, we developed a new method to compare the efficacy of an antibody after intravenous and mosquito bite inoculation of sporozoites. We then used this method to quantitatively show that the dose-dependence for an antibody targeting the malaria

parasite is different depending on the route of parasite challenge. This, combined with the fact that mosquito bite is the natural route of malaria infection suggests that, despite the technical challenges, this may be a superior model for studying the pre-erythrocytic stages in rodent models. Therefore, we recommend that future work in rodent malaria models, particularly for studying antibody responses against the pre-erythrocytic stages, be tested by mosquito bite. Our results support the idea that antibodies acting in the skin may be a critical component of vaccine-induced immunity against malaria infection.

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CURRICULUM VITAE

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Personal Data

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Education

2013	B.A.	Biochemistry & Molecular Biology (*Summa cum laude*) Cornell College, Mount Vernon, Iowa

Honors, Awards, and Fellowships

Johns Hopkins Bloomberg School of Public Health

2016 Outstanding Poster Award ($200, Annual Molecular Parasitology Meeting)
2016 Emergent BioSolutions Fellow ($5000 fellowship)
2016 Second-year Tuition Fellowship (75% tuition reduction)

Cornell College

2013 Phi Beta Kappa
2013 Dean's List – Highest Honors
2012 Tri-Beta Biology Honors Society
2012 Kao Fellow in Medical Biotechnology (highly selective Cornell Fellowship)

2011 Samuel Fellows Scholar (scholarship offered only to 10% of matriculants)

Grants Awarded

2015 Visiting Scholar Stipend (Johns Hopkins Malaria Research Institute)
2012 Dimensions – Center for the Science and Culture of Healthcare – Fund for Independent Student Research ($1500)

Research Experience

2016- **Graduate Thesis Research, Johns Hopkins Bloomberg School of Public Health**
(See below for details)
Advisor: Photini Sinnis, M.D.

2015-16 **Graduate Research Fellow, Johns Hopkins Bloomberg School of Public Health**
Optimized a CRISPR-Cas9-based strategy to generate transgenic mosquitoes. The ultimate goal is to map out the olfactory sensory systems of medically relevant mosquito vectors (*Anopheles gambiae & Aedes aegypti*) that transmit globally significant infectious diseases including Malaria, Dengue, Chikungunya, and Zika. Understanding how these insect species process sensory cues such as carbon dioxide and human odor can allow us to create effective mosquito bait traps, which are emerging tools that can be used in conjunction with bed nets to reduce disease transmission.
Advisor: Conor McMeniman, Ph.D.

2015 **Visiting Scholar, Johns Hopkins Bloomberg School of Public Health**
Optimized a mosquito bite challenge model to test the efficacy of candidate malaria vaccines for pre-clinical investigations. The efficacy of the vaccine can be determined through RT qPCR-based quantification of parasite RNA in the liver subsequent to challenge. The mosquito bite challenge model may be useful in the future for screening of candidate vaccines for malaria and other vector-borne diseases (including Dengue, Chikungunya, and Zika).

Advisor: Photini Sinnis, M.D.

2013-15 **Post-baccalaureate Research Trainee, University of Iowa**
Optimized incorporation of unnatural amino acids (with photo-crosslinking abilities) in voltage-gated Ion Channels & their auxiliary proteins to biochemically determine protein-protein interactions
Advisor: Christopher A. Ahern, Ph.D.

2012-13 **Undergraduate Researcher, University of Maryland School of Medicine**

Loading of calcium sensor into Jurkat cells, and quantification by EPR spectroscopy

Advisor: Joseph P. Y. Kao, Ph.D.

2012 **Kao Fellow in Medical Biotechnology, University of Maryland School of Medicine**

Synthesis and characterization of an EPR spectroscopy-based calcium sensor (a 10-step synthesis)

Advisor: Joseph P. Y. Kao, Ph.D.

Graduate Thesis Research, Sinnis Lab – Johns Hopkins Malaria Research Institute)

As part of the Sinnis Research Group at the Bloomberg School of Public Health (Dept. of Molecular Microbiology & Immunology), I successfully optimized a mosquito bite challenge model to test the efficacy of candidate antibody-based vaccines in mice. Furthermore, I used the model to show that antibodies targeting the rodent malaria parasite exhibit greater efficacy against mosquito bite inoculation vs intravenous inoculation of parasites. This has implications for established methods for testing candidate malaria vaccines in pre-clinical models, and for future malaria vaccine design.

Skills & Qualifications

Experimental Research Design; Training/Teaching; Data Analysis; Scientific Writing & Communication

Relevant Skills & Techniques

- Mosquito salivary gland dissections, RT qPCR, mouse dissections and I.V. & I.P. tail injections.
- Antibody purification, Immunofluorescent assays (IFAs), mouse colony management.
- Patch-clamp technique (whole-cell configuration) and two-electrode voltage clamp of *Xenopus laevis* oocytes
- Molecular biology including: various mutagenesis techniques, plasmid transformation, DNA & RNA purification, *in vitro* transcription, UV-vis spectroscopy, gel electrophoresis, flow cytometry, fluorescence microscopy, and immunoblotting
- Sterile cell culture techniques (HEK 293, CHL, Jurkats), transfections (Fugene, Lipofectamine, and Calcium Phosphate), and viral infections
- Common techniques in synthetic organic chemistry: extraction, flash column chromatography, and crystallization

- Characterization techniques in organic chemistry: TLC, GC-MS, HPLC, and NMR
- Software: GraphPad Prism, STATA, Lasergene Suite, Clampfit, Origin Pro, MiniTab, ChemDraw, and Microsoft Office

Teaching Experience

2014-15 Post-baccalaureate Research Trainee, University of Iowa
Basic laboratory skills in molecular biology (to 3 undergraduate students)

2012-13 Peer Consultant, Cornell College
Foundations in Cellular Biology, General Chemistry I & II, Organic Chemistry I & II, and Cellular & Molecular Biology

2010-11 Peer Consultant, Nashville State Community College
College Algebra, Pre-calculus I & II, Calculus I & II, General Chemistry, and Calculus-based Physics I & II

Relevant Public Health Volunteer Work

2011 **Dental Health Intern**, Mount Vernon Dental Clinic, Mount Vernon, IA
2010-11 **Emergency Services Assistant**, Saint Thomas Hospital, Nashville, TN
2009 **Patient Services Volunteer**, Beijing Chaoyang Hospital, Beijing, China

Additional Professional Development

2014 Flow Cytometry Bootcamp – ExCyte Expert Cytometry Course

Coursera

2014 Epidemiology: The Basic Science of Public Health – UNC, Chapel Hill
2013 Epigenetic Control of Gene Expression – University of Melbourne
2013 Virology I: How Viruses Work – Columbia University
2013 Vaccines – University of Pennsylvania

Relevant Publications & Presentations/Posters

2016 *Arresting Plasmodium sporozoites in the skin with IgG*, **Annual Molecular Parasitology Meeting**, Scientific Poster. Advisor: Dr. Photini Sinnis, Department of Molecular Microbiology & Immunology, Johns Hopkins Bloomberg School of Public Health.

2015 *Revealing the Macromolecular Assembly of a Sodium Channel Complex with Genetically Encoded Photochemistry*, **Health Sciences Research Week**, Scientific Poster & Oral Presentation. Advisor: Dr. Christopher A. Ahern, Department of Molecular Physiology & Biophysics, University of Iowa

2013 Intracellular loading of the calcium indicator 4,4'-diProxylBAPTA – quantitation by EPR spectroscopy, **Cornell College Student Symposium** Scientific Poster.
Advisor: Dr. Brian Nowak Thompson, Department of Biochemistry & Molecular Biology, Cornell College

2012 A Sensor for Imaging Cardiac Calcium Signaling *in vivo*, **Center for Biomedical Engineering & Technology (BioMET)** Oral Presentation. Advisor: Dr. Joseph P. Y. Kao, Department of Physiology, University of Maryland School of Medicine

Linguistic Competencies

English (native); Mandarin (working knowledge); French (working knowledge)